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ORIGINAL ARTICLE

Efficacy of a traditional unani formulation *Jawarish-e-Amla Sada* against CCl_4 induced liver toxicity in Albino Wistar rats: Comparison with silymarin treatment

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Background: *Jawarish-e-Amla Sada* (JAS); a polyherbal Unani formulation, is claimed as a potential hepatoprotective agent in traditional Unani medicine treatise. **Aims and Objectives:** Though, JAS has its composition recorded in ancient Unani texts, there have been minimal attempts to scientifically validate its hepatoprotective potential. The present work is an attempt to evaluate the hepatoprotective activity of JAS *in vivo*. **Materials and Methods:** CCl_4 intoxicated Albino Wistar rats were used to evaluate the hepatoprotective activity of JAS (0.7 g/kg and 1.0 g/kg, p.o.) and the results were compared with, silymarin. The efficacy was established on the basis of altered levels of biochemical markers and histopathological analysis of the liver tissue. **Results and Discussion:** Treatment with the aqueous slurry of JAS significantly reduced the level of biochemical markers in CCl_4 intoxicated rats. These findings were well-supported by the histopathological analysis of liver tissue. The effect shown by JAS was found to be at par with silymarin treatment. **Conclusion:** This study suggests that CCl_4 induced liver damage can be ameliorated by traditional Unani formulation JAS. This justifies the traditional claim of JAS being a hepatoprotective agent.

Key words: CCl4, hepatoprotective, *Jawarish-e-amla sada*, Unani medicine

INTRODUCTION

Liver plays a crucial role in many essential physiological processes and is vulnerable to a wide variety of toxic, microbial, circulatory, and neoplastic agents. The effectiveness of the available treatment options such as the use of corticosteroids and interferons carry the risk of adverse effect and are often too costly.[1] Therefore, many traditional and folk remedies of plant origin are being tested for their hepatoprotective activity.[2] In the past decade, there has been renewed attention and interest in the use of traditional medicine globally. Though Unani formulations are gaining global acceptance due to their amazing clinical efficacy, their scientific validity is yet to be documented.[3] *Jawarish-e-Amla sada* (JAS); a semisolid traditional Unani formulation of six medicinal herbs [Table 1], is commonly used in the weakness of stomach, liver, and heart. JAS is also prescribed to treat palpitation, flatulence in stomach, and biliary diarrhoea.[4]

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Recently, we have reported the parameters used for the standardisation of JAS.[5] However, the use of JAS as a hepatoprotective agent is yet not reported. Pericarp of *Emblica officinalis* (the major ingredient of JAS) and gallic acid [Figure 1, the major phytochemical constituent of *E. officinalis*] are scientifically reported for their use in hepatic dysfunction.[6] Based on these facts, the present work was undertaken to evaluate JAS as an alternative cure for hepatic injuries in CCl_4 induced liver toxicity in Albino Wistar rats and the results have been statistically compared with the known hepatoprotectant silymarin. JAS was standardised in terms of its gallic acid content prior to its administration to CCl_4 intoxicated rats. The study justifies the traditional claim of JAS in the clinical treatment of hepatic disorders.

MATERIALS AND METHODS

Preparation of *Jawarish-e-Amla Sada* and Quality Evaluation

Jawarish-e-Amla sada was prepared as per the method reported by our group[5] using the raw materials of pharmacopoeial quality and quantity and standardised in terms of gallic acid content.

Chemicals and Reagents

Silybon tablets (silymarin as silybon 70 mg, Batch no.: SIAD0025, Microlabs, Ltd.) and CCl_4 (GR grade,

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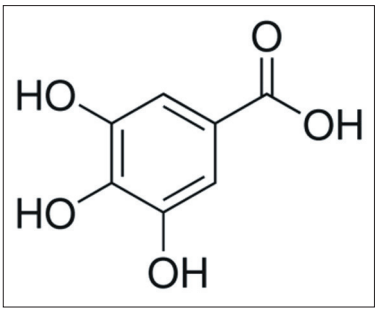

Figure 1: Structure of gallic acid

Batch no. IG8G580178, Merck Specialties Pvt., Ltd) were procured from the market. All other chemicals used were of analytical grade.

Animals

Adult Albino Wistar female rats (200–250 g) were procured from Haffkine Biopharmaceuticals, Parel, Mumbai. The animals were maintained under standard laboratory conditions at an ambient temperature of 25°C ± 2°C with 12 h light and dark cycle in an animal house with standard facility under Committee for the Purpose of Control and Supervision of Experiments on Animals approval. They were fed with rat feed and water *ad libitum*.

Preparation of the Aqueous Slurry

Aqueous slurry was prepared by mixing appropriately weighed JAS in distilled water. The animals were dosed individually as per their body weights.

Assessment of Hepatoprotective Activity Against CCl_4 Induced Liver Intoxication

Hepatic injury induced by CCl_4 is commonly used for the screening of hepatoprotective drugs and the extent of hepatic damage is assessed by the level of released biochemical markers.[7] In this study, Albino Wistar rats were randomly divided into six groups of six animals each [Table 2].

The animals were fasted overnight before the initiation of the study. Animals from Group I received an i.p. injection of liquid paraffin/animal (0.5 ml) on the day 1 of the study and were treated as normal control. Those from Groups II, III, IV, V and VI; received an i.p. injection of CCl_4 in 0.5 ml liquid paraffin/animal (1.2 ml/kg) on day 1 of study.[8] The animals from Groups I, II, and III received an oral dose of distilled water (2 ml) once daily. Animals from Group IV received silymarin (suspended in distilled water 2 ml, 0.07 g/kg, p.o.) 1 h post-induction on day 1 and daily thereafter.[7,9,10] The animals from Group V and VI received aqueous slurry of JAS (0.7 g/kg and 1.0 g/kg, respectively, p.o.) 1 h post-induction on day 1 and daily thereafter.

Prior to sacrifice, blood (2 ml) was collected from each animal under light ether anaesthesia through retro-orbital plexus in non-heparinised vials, centrifuged at 5000 rpm for 8 min. The serum was separated and analysed for the biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total bilirubin (BIL), cholesterol (CHO), and triglycerides (TG). The animals from Group I, II, IV, V, and VI were sacrificed on day 4. Animals from Group III were sacrificed on day 7 for comparative evaluation of natural recovery. Daily record of body weight, food, and water intake was also maintained.

Table 1: Formula composition of JAS

Ingredients		Quantity (g or mL)
Unani name	**Botanical identity**	
Amla Khushk	Pericarp of *Emblica officinalis* L.	50
Post-e-Turanj	Fruit peel of *Citrus medica* L.	10
Sandal Safaid	Stem of *Santalum album* L.	10
Mastagi	Gum resin of *Pistacia lentiscus* L.	5
Dana Heel Khurd	Seeds of *Elettaria cardamomum* L. Maton	5
Gulnar farsi	Flowers of *Punica granatum* L.	5
Qand safaid	Sugar	375
Asal	Honey	375
Aab	Water	375

JAS – *Jawarish-e-Amla Sada*

Table 2: Group details and dosage regimen

Group	Details
I	Normal control
II	CCl4 control (1.2 ml/kg)
III	CCl4 control as natural recovery group
IV	CCl4 control treated with daily dose of silymarin (0.07 g/kg)
V	CCl4 control treated with daily dose of JAS aqueous slurry (0.7 g/kg)
VI	CCl4 control treated with daily dose of JAS aqueous slurry (1.0 g/kg)

JAS – *Jawarish-e-Amla Sada*

During autopsy, liver was excised, rinsed in saline, blotted and weighed. A small piece of liver tissue from the largest lobe was cut, fixed into Bouin's fixative and processed for routine haematoxylin and eosin staining. Percent protection in biochemical parameters was calculated as 100× (values of CCl_4 Control-values of sample)/(values of CCl_4 control-values of vehicle).[2]

Statistical Analysis

Values are expressed as mean ± standard error and statistically analysed for significance with the other groups using GraphPad Prism 5 software (GraphPad Software, Inc., California, USA). $P \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Jawarish-e-Amla sada has been traditionally prescribed for the treatment of hepatic disorders and hence is commercially

marketed by various brands. Therefore, the present study was undertaken to evaluate the aqueous slurry of JAS as an alternative cure for hepatic injuries in CCl_4 induced animal model.

CCl_4 induction causes elevated level of SGOT, SGPT and other liver biomarkers in circulation.[11] It also results in classical fatty liver as indicated by a significant increase in CHO.[9,10] In this study, CCl_4 induction control (Group II) showed marked increase in all biochemical parameters analysed (SGOT, SGPT, TB, CHO and TG) [Table 3]. Elevated levels of the biochemical parameters indicate damage to the liver by CCl_4 induction. This was evident from the findings of histopathological studies too wherein CCl_4 induction resulted in severe loss of hepatic architecture in the form of intense peripheral and central vein necrosis, fatty changes, etc., when compared to normal control group [Figure 2a, 2b].

Rats treated with the established modern medicine silymarin showed marked recovery in the biochemical parameters (SGOT, SGPT, TB, CHO, and TG) and offered percentage protection of 47.24%, 72.34%, 94.67%, 91.52% and 87.80%, respectively [Table 3]. Histopathological findings supported the biochemical data as the treated animals showed near normal hepatic architecture with a mild degree of necrosis, which signifies its protective effect and recovery [Figure 2(d)].

Animals treated with aqueous slurry of JAS at two different doses 0.7 and 1.0 g/kg (equivalent to 0.2 and 0.6 mg gallic acid, respectively) showed a significant reduction in the levels of biochemical parameters. At low dose, the percentage protection offered by JAS was found to be 29.43%, 56.03%, 63.82%, 80.65% and 66.95% for SGOT, SGPT, TB, CHO and TG, respectively. Similarly, high dose of JAS showed the percentage protection of 42.51%, 79.13%,

Table 3: Effect of silymarin and JAS on SGOT, SGPT, BIL, CHO and TG in CCl_4 induced liver toxicity in rats

Groups	Biochemical parameters (percentage protection)				
	SGOT in U/L	SGPT in U/L	BIL in×10 mg/dL	CHO in U/L	TG U/L
Normal control	33.45±1.39	29.28±1.89	51.33±2.31	48.49±1.71	73.96±2.65
CCl_4 control	89.54±1.67*	82.36±2.37*	82.67±3.18*	121.62±2.80*	129.30±5.12*
CCl_4 recovery	71.23±1.76 (19.10)	62.36±2.22 (37.68)	71.33±2.08 (36.18)	97.68±3.34 (32.74)	117.75±3.46 (20.87)
Silymarin	44.26±2.31* (47.24)	43.96±2.65* (72.34)	53.00±1.46* (94.67)	54.69±1.95* (91.52)	80.71±3.81* (87.80)
JAS (0.7 g/kg)	61.33±1.43* (29.43)	52.62±1.48* (56.03)	62.67±1.84* (63.82)	62.64±3.24* (80.65)	92.25±2.91* (66.95)
JAS (1.0 g/kg)	48.79±2.38* (42.51)	40.36±1.82* (79.13)	54.33±2.08* (90.43)	50.14±2.01* (97.74)	82.19±2.47* (85.13)

All values are mean±SE; *n*=6. *Represents *P*≤0.05. SE – Standard error; JAS – *Jawarish-e-Amla Sada*; SGOT – Serum glutamate oxaloacetate transaminase; SGPT – Serum glutamate pyruvate transaminase; BIL – Bilirubin; CHO – Cholesterol; TG – Triglycerides

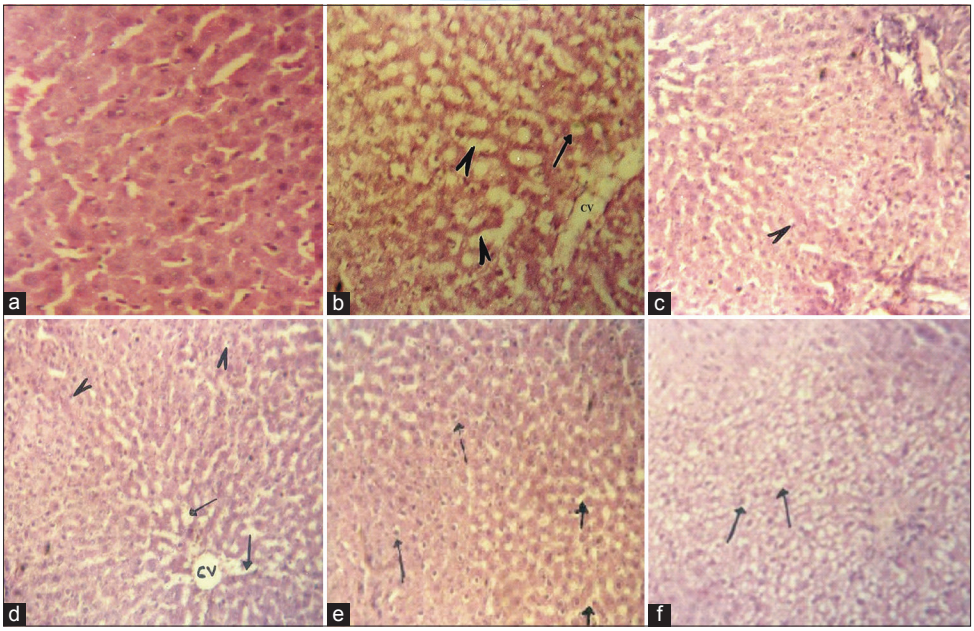

Figure 2: Effect of silymarin and *Jawarish-e-Amla sada* (JAS) on histopathological changes in the liver of rats in CCl_4 induced liver toxicity in rats. (a) Normal control; (b) induction control (liver of CCl_4 intoxicated animal); (c) liver of CCl_4 intoxicated animal after 7 days of natural recovery; (d) liver of CCl_4 intoxicated animals treated with silymarin; (e) and (f) Liver of CCl_4 intoxicated animals treated with JAS 0.7 g/kg and 1.0 g/kg, respectively. CV – Central vein, Arrows – dilated sinusoids, Arrowheads – vacuolated hepatocytes

90.43%, 97.74% and 85.13% for SGOT, SGPT, TB, CHO and TG, respectively [Table 3].

The histopathological results of the rats treated with JAS showed marked recovery in the hepatic architecture and reduction in liver damage and cellular necrosis when compared to the histopathological findings of CCl_4 induction control. JAS slurry at higher dose showed better results in terms of percent protection and histological findings when compared with the lower dose [Figure 2e and f]. Significant reduction in the level of biochemical parameters suggests the possible role of silymarin or JAS in stabilisation of plasma membrane and repair of hepatic tissue damage caused by CCl_4 induction.[12] Noticeable reduction in the average food and water intake of the animals coupled with decrease in the body weight post CCl_4 induction indicated its toxic response, whereas significant enhancement in these characters after the treatment with JAS indicated its protective potential (data not shown). This finding was found in compliance with the published reports.[6,7,9,10]

Animals of group III sacrificed on day 7 showed 19.10%, 37.68%, 36.18%, 32.74% and 20.87% reduction in SGOT, SGPT, BIL, CHO and TG level, respectively. Liver sections of the animals in natural recovery group showed similar results as of induction group [Figure 2c]. From these observations, it can be interpreted that the animals did not show significant natural reduction in biochemical parameters even after the extended recovery period of 7 days. This confirms the significant enhancement of recovery rate by silymarin and JAS.

CONCLUSION

The present study elucidates the dose-dependent hepatoprotective activity of JAS in comparison with the established drug silymarin in CCl_4 intoxicated rats. The protection provided by JAS was found at par with silymarin treatment. Findings of the present work also support the traditional use of JAS in the treatment of hepatoprotective dysfunction. The hepatoprotective potential of JAS may be attributed to its phytochemical constituents (especially gallic acid). Thus, the present data provide a basis for use of JAS as a suitable Unani medicine for the treatment of CCl_4-induced liver damage. However, to rationalize the dose of JAS for therapeutic use, more work needs to be carried out.

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REFERENCES

1. Shamsi-Baghbanan H, Sharifian A, Esmaeili S, Minaei B Hepatoprotective herbs, avicenna viewpoint. Iran Red Crescent Med J 2014;16:e12313.
2. Ganga Rao B, Venkateswara Rao Y, Mallikarjuna Rao T. Hepatoprotective activity of *Spillanthes acmella* extracts against CCl_4-induced liver toxicity in rats. Asian Pac J Trop Dis 2012;2:S208-11.
3. Shailajan S, Menon S, Sayed N, Joshi H, Tiwari B. Phytochemical, safety and pharmacokinetic profile of *Jawarish-e-Bisbasa*: A potent Unani medicine for gastric disorders. Afr J Tradit Med 2012;7:168-77.
4. National Formulary of Unani Medicine. 1st ed. New Delhi: Controller of Publications; 1983. p. 97-8.
5. Shailajan S, Swar G, Matani A, Tiwari B. Quality evaluation and standardization of a traditional Unani formulation *Jawarish-e-Amla Sada*. Int J Green Pharm 2015;9:21-5.
6. Malar HL, Bai SM. Hepatoprotective activity of *Phyllanthus emblica* against paracetamol induced hepatic damage in Wister Albino rats. Afr J Basic Appl Sci 2009;1:21-5.
7. Shailajan S, Joshi M, Tiwari B. Hepatoprotective activity of *Parmelia perlata* (Huds.) ach. Against CCl_4 induced liver toxicity in Albino Wistar rats. J Appl Pharm Sci 2014;4:70-4.
8. Koh PH, Mokhtar RA, Iqbal M. *Andrographis paniculata* ameliorates carbon tetrachloride (CCl (4))-dependent hepatic damage and toxicity: Diminution of oxidative stress. Redox Rep 2011;16:134-43.
9. Shailajan S, Chandra N, Sane RT, Menon S. Effect of *Asteracantha longifolia* nees. Against galactosamine induced liver dysfunction in rats. Toxicol Int 2007;14:7-13.
10. Shailajan S, Chandra N, Sane RT, Menon S. Effect of *Asteracantha longifolia* Nees. Against CCl4 induced liver dysfunction in rat. Indian J Exp Biol 2005;43:68-75.
11. Contran RS, Kumar V, Robbins SL. Pathologic Basis of Diseases. 5th ed. Philadelphia: WB Saunder Company; 1994. p. 178-89.
12. Anusha M, Venkateswarlu M, Prabhakaran V, Taj SS, Kumari BP, Ranganayakulu D Hepatoprotective activity of aqueous extract of *Portulaca oleracea* in combination with lycopene in rats. Indian J Pharmacol 2011;43:563-7.

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